53. A method for introducing DNA sequences encoding SDI-1 into human cells in vitro comprising infecting a target cell population with a retroviral particle produced by the producer cell line of Claim 50.---

## REMARKS

Information Disclosure Statement

An Information Disclosure Statement (IDS) is being filed concurrently herewith. Entry of the IDS is respectfully requested.

Rejection of Claims 1-4, 8-11, 13-16, 19-23, 26-28, 31 and 32 under 35 U.S.C. §112, first paragraph

Claims 1-4, 8-11, 13-16, 19-23, 26-28, 31 and 32 are rejected under 35 U.S.C. §112, first paragraph "as containing subject matter which was not described in the specification in such a way as to enable one skill in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention for reasons of record set forth in the office action of 5-24-99" (Office Action, page 3).

The Examiner cites Feldman and Crystal as teaching that "it is unpredictable whether therapeutic effects can be obtained against restenosis, cancer or any other disease using gene therapy because of the low efficiency of cells expressing the transgene, low transfection efficiency, the lack of target specificity, the lack of sustained expression and the lack of predictable expression of genes in humans" (Office Action, page 3). It is the Examiner's opinion that the "specification does not provide adequate guidance correlating the results *in vitro* to results obtained *in vivo* in such a way that one of skill would have a reasonable expectation in obtaining a therapeutic level of expression of SDI-1 such that cancer or restenosis could be treated" (Office Action, page 4).

Applicants respectfully disagree. In the specification as filed, Applicants demonstrate that a human bladder carcinoma derived cell line transfected with a gene encoding SDI-1 "showed significantly more cells in $G_0/G_1$ when grown in the presence of Dex after serum

starvation (61%) than in the absence of SDI-1 expression (50.6%)" (specification, page 26, lines 20-22). It is reasonable to expect based on Applicants' data that producer cells stably transfected with a retroviral particle comprising a DNA sequence encoding SDI-1, retroviral particles produced by such a cell line and capsules which encapsulate such producer cell lines can be used to treat diseases or disorders responsive to the anti-proliferative activity of SDI-1. Evidence of this is provided in the art made of record. The Examiner states that Applicants' claimed method of treatment are obvious in light of the teachings of Nabel *et al.*, Miller *et al.* and Price *et al.* For example, Nabel *et al.* showed that expression of p21 inhibted vascular cell proliferation and induced cell cycle arrest *in vitro* (Nabel *et al.*, column 7, line 59 - column 8, line 60) and that these *in vitro* results correlated to results they received *in vivo* (Nabel *et al.*, column 8, line61 - column 11, line 7). Furthermore, as noted by the Examiner, Miller *et al.* teach that "retroviruses can be used in vivo" and Price *et al.* Demonstrate that "retroviral particles can be used to deliver gene of interest *in vivo*" (Office Action, page 9). Indeed, Miller *et al.* clearly teach that retroviral vectors "will be useful for the treatment of humans" (Miller *et al.*, page 989, column 3). Clearly Applicants have provided an enabling disclosure for the full scope of the claimed invention.

The Examiner states that "Applicants have enabled one of skill to determine amino acids 1-71 or 42-58 of the human SDI-1 gene disclosed in WO 95/06415 but not any other human SDI-1, WAF-1, CIP1 PIC or p21 sequence disclosed in the art" (Office Action, page 5). The Examiner further states that "one of skill would not be able to determine what applicants consider amino acids 1-71 or 42-58 of SDI-1 other than the sequence disclosed in WO 95/06415" (Office Action, page 5). The Examiner further states that the "specification has not taught any method to identify functionally useful analogues or fragments of the human SDI-1 gene taught in WO 95/06415" and that "[s]uch methods are not routine and are considered essential to determine functionally equivalent analogues or fragments as claimed" (Office Action, page 5).

Applicants respectfully disagree. Making functional fragments of a known protein and determining functional analogues of a known protein for which functional analogues are already known, are straightforward and reasonably predictable (absolute predictability is not required). For example, one of ordinary skill in the art can predict that deleting a single amino acid residue from one of the ends of a SDI-1 protein or one of its known analogues would likely produce a

protein that retains biological activity, whereas deleting 95% of the residues would probably not. There is a substantial amount of readily available information in this field of art that provides a solid basis for making reasonably accurate predictions about protein structure and function. Furthermore, those of ordinary skill in the art are guided by sequence conservation. In addition, any fragment or suspected analogue of SDI-1 can be analyzed for activity using known methods. In the specification as filed, Applicants provide assays for doing so. (See Example 5). Clearly, Applicants have provided an enabling disclosure for identifying fragments and analogues of SDI-1 which inhibit cell proliferation.

The Examiner states that the "only disclosed use for encapsulated cells and pharmaceutical compositions in the specification is for administering the cells *in vivo* to obtain therapeutic effects" (Office Action, page 5).

Applicants respectfully disagree. As indicated above, claims directed to pharmaceutical compositions have been canceled. However, Applicants teach that the DNA sequence encoding SDI-1 can be introduced into cells *in vitro* comprising infecting a target cell population with the claimed retroviral particles (Claim 26). Clearly encapsulated cells comprising a packaging cell line which is used to produce the retroviral particles can also be used to introduce DNA encoding SDI-1 into cells *in vitro*.

The Examiner states that "Claim 2 recites the new limitation of a vector which carries a DNA sequence" and that "[v]ectors may comprise DNA but do not carry DNA" (Office Action, page 6).

Claim 2 has been amended to replace the term "carry" with the term "comprise".

Applicants have provided an enabling disclosure for the full scope of the claimed invention.

Rejection of Claims 31 and 32 under 35 U.S.C. §112, second paragraph

Rejection of Claims 31 and 32 under 35 U.S.C. §112, second paragraph "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention" (Office Action, page 6). The Examiner states that "the claims refer to claim 28 which is limited to delivery of a packaging cell line", and "[i]t is unclear

whether applicants intend to claim administering retroviral particles or retroviral packaging cell lines" (Office Action, page 6).

Claim 27 has been amended to depend from Claim 1. Such amendment makes clear that retroviral particles are administered.

Rejection of Claims 1-4 and 9 under 35 U.S.C. §102(b)

Claims 1-4 and 9 are rejected under 35 U.S.C. §102(b) as being anticipated by Tsang *et al.* "for reasons of record set forth in the office action of 5-24-99" (Office Action, page 7). The Examiner notes Applicants' position that Tsang *et al.* do not teach SDI-1 protein which inhibits cell proliferation, but states that "Claims 1-4 and 9 do not require that the protein inhibit cell proliferation" (Office Action, page 7).

As amended, Claim 1 is drawn to a method for producing a recombinant retroviral particle, said particle comprising a DNA sequence encoding SDI-1, a functional analogue, or a fragment thereof wherein the SDI-1 or functional fragment or functional analogue thereof inhibits cell proliferation.

Tsang *et al.* do not anticipate Applicants' claimed invention, particularly as amended.

Rejection of Claims 1-4, 8, 13, 14, 19, 26-28, 31 and 32 under 35 U.S.C. §103(a)

Claims 1-4, 8, 13, 14, 19, 26-28, 31 and 32 are rejected under 35 U.S.C. §103(a) as being unpatentable over Miller *et al.* or Price *et al.* in view of Nabel *et al.*(U.S. Patent No. 5,863,904). The Examiner cites Miller *et al.* and Price *et al.* as evidence that "[m]ethods of making retroviral particles were well known to the ordinary artisan at the time of filing of the instant invention" (Office Action, page 8). The Examiner notes that Miller *et al.* and Price *et al.* "do not teach the method of making retroviral particles encoding SDI-1 or producer cells transfected with a retroviral vector encoding SDI-1" (Office Action, page 8). The Examiner cites Nabel *et al.* as teaching the use of "retroviral vectors comprising the gene encoding human p21 to accumulate cells in $G_0/G_1$" and that SDI-1 is equivalent to p21. It is the Examiner's opinion that:

> [i]t would have been obvious to one of ordinary skill at the time the instant invention was made to produce the retroviral vector taught by Nabel et al. using the method taught by Miller et al. or Price et al. One of ordinary skill would have

> recognized that the retroviral vector taught by Nabel et al. encoding p21 had to be produced using methods such as those taught by Miller et al. or Price et al. One of ordinary skill would have been motivated to produce retroviral vectors encoding p21 as taught by Nabel et al. using the methods taught by Miller et al. or Price et al. to treat disease. Additional motivation is provided by Miller et al. by stating the retroviruses can be used *in vivo* . . . and by Price et al. by demonstrating the retroviral particles can be used to deliver genes of interest *in vivo*. . . The method of administering retroviral particles . . . is obvious in view of the teachings of Nabel et al. and Miller et al. because Price et al. suggests using retroviral particles to deliver genes of interest and because delivery of retroviral particles was commonly performed *in vivo*. One of ordinary skill would have had a reasonable expectation of success in obtaining the claimed invention because methods of producing retroviral vectors, packaging cells and methods of administering retroviral particles were well known at the time of filing (Office Action, pages 9-10).

Applicants respectfully disagree. As discussed in more detail below, Applicants teach *stably transfecting* a producer cell line with a retroviral vector (an RNA vector) comprising DNA encoding SDI-1 wherein retroviral particles which carry the SDI-1 gene *are released by budding and the producer cell line remains intact and can perform cell division*. In contrast, Nabel *et al.* teach *transiently transfecting* a producer cell line with an adenoviral vector (a DNA vector) comprising DNA encoding SDI-1 wherein viral particles *are released by lysis with the result that the producer cells are killed*. Stable transfection requires that the producer cells can divide.

Applicants' claimed invention relates to a method for producing a recombinant retroviral particle, said particle comprising a DNA sequence encoding SDI-1, a functional analogue, or a fragment thereof wherein the SDI-1 or functional fragment or functional analogue thereof inhibits cell proliferation, comprising *stably transfecting* a producer cell with *a retroviral vector* comprising the DNA sequence, said producer cell additionally harboring at least one DNA construct coding for proteins required for said retroviral vector to be packaged. Generation of RNA viruses such as retroviruses initially involves the transcription of the recombinant DNA vector into RNA. Subsequently, the RNA molecules are packaged into viral particles and released by *budding* from the packaging cells. The proteins for packaging of these viruses are provided by packaging constructs included in the packaging cell line.

The combined teachings of Miller *et al.*, Price *et al.* and Nabel *et al.* do not teach *stably* transfecting packaging cells (*i.e.*, 293 cells) with a retroviral vector comprising DNA encoding SDI-1 which additionally harbors at least one DNA construct coding for proteins required for said retroviral vector to be packaged, wherein RNA molecules are packaged into viral particles and released by *budding* from the packaging cells.

Miller *et al.* "designed a set of retroviral vectors which cannot yield helper virus by homologous recombination with the retroviral genome present in the packaging cells, and include mutations to block viral protein synthesis" (Miller *et al.*, page 980, column 3). Price *et al.* demonstrate that "retroviruses that encode the β-gal gene can be successfully introduced into the rat retina and mark cells such that they can easily be detected histochemically" (Price *et al.*, page 156, column 2). Nabel *et al.* produced an adenoviral vector (*i.e.*, a DNA vector) comprising the p21 gene and the genes required for synthesis of packaging proteins and in which the E1A and A1B replicators and activators of adenoviral gene expression were deleted. The deleted E1A and E1B were provided by the 293 packaging cell line used for propogation of recombinant adenoviruses (Nabel *et al.*, column 6, lines 22-34). With DNA virus-derived vectors, the packaging cells are *lysed* upon release of the DNA viruses.

> Noting Applicants' position in the previously filed Amendment, the Examiner states that:
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> Applicants argument is not persuasive. The 293 packaging cells of Nabel et al. are equivalent to 293 cells taught in the specification (page 16, line 5) and can be used to make retroviral vectors as taught by Nabel et al. In addition, the claims do not require release of the retroviral particle by budding (Office Action, page 12).

Applicants respectfully disagree. The claims do require release of retroviral particles by budding since, by definition, "retroviral particles" are released from a producer cell via budding. Retroviral particles are *not* released from a cell via any other process (*e.g.*, lysis). Additionally, it is by virtue of this budding process that allows for a method of producing a recombinant retroviral particle comprising a DNA sequence encoding SDI-1 wherein a producer cell, which harbors at least one DNA construct coding for proteins required for said retroviral vector to be packaged, is stably transfected with a retroviral vector comprising the DNA sequence. Stable transfection is not possible using the DNA vectors of Nabel *et al.* because the cells are lysed, or killed, after release of the viral particles. Thus, it is not true that Nabel *et al.* teach "successful

division of 293 packaging cells expressing SDI-1" (Office Action, pages 12-13), because, in contrast to Applicants' claimed invention, cell division cannot occur after release of the viral particles of Nabel *et al.*; the producer cells of Nabel *et al.* are destroyed upon release of viral particles.

Furthermore, as pointed out in the previously filed Amendment, a person of skill in the art would not be motivated to combine the teachings of Nabel *et al.* with the teachings of Price *et al.* to produce Applicants' claimed invention because one of skill would not expect that a *stably transfected producer cell line comprising a retroviral genome which encodes the SDI-1* could be produced. Briefly, SDI-1 is known to inhibit cell proliferation and DNA synthesis, and thus, prevent cell division. Accordingly, a person of skill in the art would not expect to get a stable population of retrovirus producing cells by stable integration of a recombinant retroviral vector comprising the SDI-1 gene. Rather, a person of skill in the art would expect that after integration of the retroviral vector into the genome of the packaging cell, division of the cell would be inhibited by virtue of the expressed SDI-1 protein, and thus, stable daughter cells which produce RNA-virus would not be generated. Surprisingly, Applicants have shown that stable populations of recombinant retroviral particle producing cells stably transfected with a retroviral vector comprising SDI-1 are generated.

The Examiner states that this argument is not persuasive because "Nabel et al. does not teach SDI-1 expression prevents 293 packaging cell division or production of viral particles" (Office Action, page 12). Nabel *et al.* teach production of viral particles by the packaging cell line, however, Nabel *et al. do not and cannot* teach expression of 293 cells after release of the viral particle because the 293 cells were lysed.

Clearly, the teachings of Miller *et al.*, Price *et al.* and Nabel *et al.*, either alone or in combination, do not teach or even suggest a method for producing a recombinant retroviral particle, said particle comprising a DNA sequence encoding SDI-1, a functional analogue, or a fragment thereof wherein the SDI-1 or functional fragment or functional analogue thereof inhibits cell proliferation, comprising *stably transfecting* a producer cell with *a retroviral vector* comprising the DNA sequence, said producer cell additionally harboring at least one DNA construct coding for proteins required for said retroviral vector to be packaged.

The teachings of Miller *et al.*, Price *et al.* and Nabel *et al.* do not render obvious Applicants' claimed invention. Withdrawal of the rejection is respectfully requested.

Rejection of Claims 1 and 9-11 under 35 U.S.C. §103(a)

Claims 1 and 9-11 are rejected under 35 U.S.C. §103(a) as being unpatentable over Miller *et al.* or Price *et al.* in view of Nabel as applied above, and further in view of Haertig *et al.* The Examiner states that Miller *et al.* and Price *et al.* "teach methods of producing retroviral particles comprising transfecting packaging cells PA317 with a retroviral vector encoding β-gal with a 5' LTR . . . or packaging cells NIH 3T3", but notes that Miller *et al.* and Price *et al.* "do not teach the method of making retroviral particles encoding SDI-1 or producer cells transfected with a retroviral vector encoding SDI-1" (Office Action, page 10). The Examiner cites Nabel *et al.* as teaching the use of "retroviral vectors comprising the gene encoding human p21 to accumulate cells in $G_0/G_1$" and that "SDI-1 is equivalent to p21" (Office Action, pages 10-11). The Examiner notes that Miller *et al.*, Price *et al.* and Nabel *et al.* "do not teach using the MMTV regulatory elements" (Office Action, page 11). The Examiner cites Haertig *et al.* as teaching the "MMTV regulatory elements which can be used to created a chimeric retroviral vector to obtain mammary cell-specific expression of the gene of interest" (Office Action, page 11). It is the Examiner's opinion that:

> "[i]t would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the method of making a retroviral particle taught by Miller et al., Price et al. and Nabel et al. with the MMTV regulatory elements of Haertig et al. One of ordinary skill would have been motivated to deliver retroviral vectors to breast cancer as suggested by Nabel et al. . . . using the MMTV regulatory elements taught by Haertig et al. because Haertig et al. teach the MMTV regulatory elements are mammary specific. One of ordinary skill would have been motivated to target breast tissue to improve p21 expression in breast cancer and to obtain a greater therapeutic effect. One of ordinary skill would have had a reasonable expectation of success in obtaining the method claimed using the teachings of Miller et al. Price et al., Nabel et al. and Haertig et al. because methods of producing retroviral particles and of methods of inserting promoters to retroviral vectors were common at the time of filing" (Office Action, page 11).

Applicants respectfully disagree. As discussed above, the teachings of Miller *et al.*, Price *et al.* and Nabel *et al.*, either alone or in combination, do not teach a method for producing a recombinant retroviral particle, said particle comprising a DNA sequence encoding SDI-1, a functional analogue, or a fragment thereof wherein the SDI-1 or functional fragment or functional analogue thereof inhibits cell proliferation, comprising *stably transfecting* a producer cell with *a retroviral vector* comprising the DNA sequence, said producer cell additionally harboring at least one DNA construct coding for proteins required for said retroviral vector to be packaged.

Haertig *et al.* do not provide what is lacking in the combined teachings of the Miller *et al.*, Price *et al.* and Nabel *et al.* references. Haertig *et al.* "show that MMTV expression is regulated by cell density in GR mouse mammary cells but not in NIH 3T3 mouse fibroblasts"...and that this "effect is mediated by binding sites in the HRE for the transcription factors OTFI and CTF/NFI" (Haertig *et al.*, page 814, column 1). Haertig *et al.* do not provide any motivation to produce a recombinant retroviral particle, said particle comprising a DNA sequence encoding SDI-1, a functional analogue, or a fragment thereof wherein the SDI-1 or functional fragment or functional analogue thereof inhibits cell proliferation, comprising *stably transfecting* a producer cell with *a retroviral vector* comprising the DNA sequence, said producer cell additionally harboring at least one DNA construct coding for proteins required for said retroviral vector to be packaged. Furthermore, there is nothing in the Haertig *et al.* reference that would lead one of skill to expect that a *stably transfected producer cell line comprising a retroviral genome which encodes the SDI-1* could be produced

The teachings of Miller *et al.*, Price *et al.*, Nabel *et al.* and Haertig *et al.* do not render obvious Applicants' claimed invention. Withdrawal of the rejection is respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (781) 861-6240.

Respectfully submitted,

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